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ARTICLE

Comparison of wound strength, histologic, and aesthetic outcomes after microsurgical versus conventional skin closure in a rat model

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ABSTRACT

The purpose of this study was to compare the healing, strength, and cosmetic outcome of linear incisions after repair with the naked eye, surgical loupes, or a surgical microscope. Two parallel incisions were made on the dorsal skin of Sprague–Dawley rats ($n = 36$) and the rats randomized into four groups. A single surgeon repaired the incisions using 5-0 poliglecaprone in a running subcuticular pattern using the naked eye (Group I), surgical loupes with 2.5 \times magnification (Group II), surgical microscope with 5–10 \times magnification (Group III), and 6-0 poliglecaprone with a surgical microscope (Group IV). Rats were sacrificed at 1, 3, and 6 weeks. At each time point, the tensile strength of each closure was assessed. Macroscopic outcomes were evaluated using the Vancouver Scar Scale (VSS) and histology assessed by a blinded observer. Microscope closure took significantly longer than closure with the naked eye ($p < 0.05$). There was no significant difference in tensile strength or VSS ratings between the closure methods at any of the time points. On histopathologic analysis, there were a greater number of inflammatory cells and fibroblasts in the 6-0 microscope closure group versus the naked eye closure group at week 3 ($p \leq 0.05$). In conclusion, wound repair under magnification did not yield a significant difference in cosmesis or wound tensile strength, but did increase operative time. Moreover, there was a trend toward increased inflammation with microscope-assisted closures, perhaps due to the increased suture burden.

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Scar; scar strength; scar aesthetics; microscope; rat model

Introduction

One aspect of a successful operation is a scar that is cosmetically acceptable to both the patient and surgeon. Too often, an unsightly surgical scar can take the focus away from an otherwise excellent result. Patients and surgeons strive to minimize surgical scarring with various techniques. Intra-operatively, excellent wound alignment, proper wound eversion and strategies that minimize tension can all maximize the potential for a fine line and an acceptable scar [1–4]. Other aspects of closure that have already been subjected to extensive study include suture type [5,6] and size [7], suture pattern [8–10], and other non-suture based closure techniques such as staples [11–13] or skin adhesives [14].

The benefit of magnification for accurate identification of anatomic structures is well established [15–17]. However, routine skin closures are almost always accomplished with the naked eye. Certainly, naked eye closure is often adequate for the average surgical incision. However, what is not known is whether the cosmetic outcome of an incision could be improved with further magnification. If better cosmetic outcomes can be achieved using loupes or even a surgical microscope, then surgeons should be able to offer this as a gold standard. While the efficiency of such closures would certainly be scrutinized in today's insurance-based medical system, decreased need for scar revisions may balance this consideration. Additionally, patients may be willing to incur additional costs for these time-consuming techniques, if proven beneficial.

In this study, we used a murine model to compare different magnification-assisted skin laceration repairs in terms of wound strength, histologic, and aesthetic outcomes.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee at David Grant USAF Medical Center, Travis Air Force Base, California. All animal care and use was in compliance with the Guide for the Care and Use of Laboratory Animals in a facility accredited by AAALAC.

Thirty-six 8- to 12-week-old male Sprague–Dawley outbred rats (Envigo, Hayward, CA) weighing 310–350 g were used for the study (Figure 1). Animals were anesthetized using 5% isoflurane in 100% oxygen in an induction chamber, and anesthesia was maintained with 2–4% isoflurane in 1 l/min of oxygen via face-mask. Prior to incision, Buprenorphine sustained release (ZooPharm, Windsor, CO) 0.12 mg/kg was injected at the planned incision line. A warming blanket set at 104 °F was used to maintain the rat's body temperatures during the procedure.

After adequate anesthesia was achieved, the dorsal skin of each subject was clipped and prepped in a sterile fashion. In accordance with the protocol designed by Souza et al. [18], a 3-cm full thickness longitudinal incision was made on either side of the vertebral column (72 total incisions). The incisions were then randomized into four groups and closed using 5–0 poliglecaprone (Monocryl, Ethicon, Somerville, NJ) in a running subcuticular pattern by either naked eye (Group I), surgical loupes with 2.5 \times magnification (Group II), or by a surgical microscope with 5–10 \times magnification (Group III). In the remaining group (Group IV), incisions were closed with 6–0 poliglecaprone (Monocryl, Ethicon) in a running subcuticular pattern using a surgical microscope with 5–10 \times magnification. We elected to include group IV due to a prior study by Yang et al. [7] that indicated suture size may have

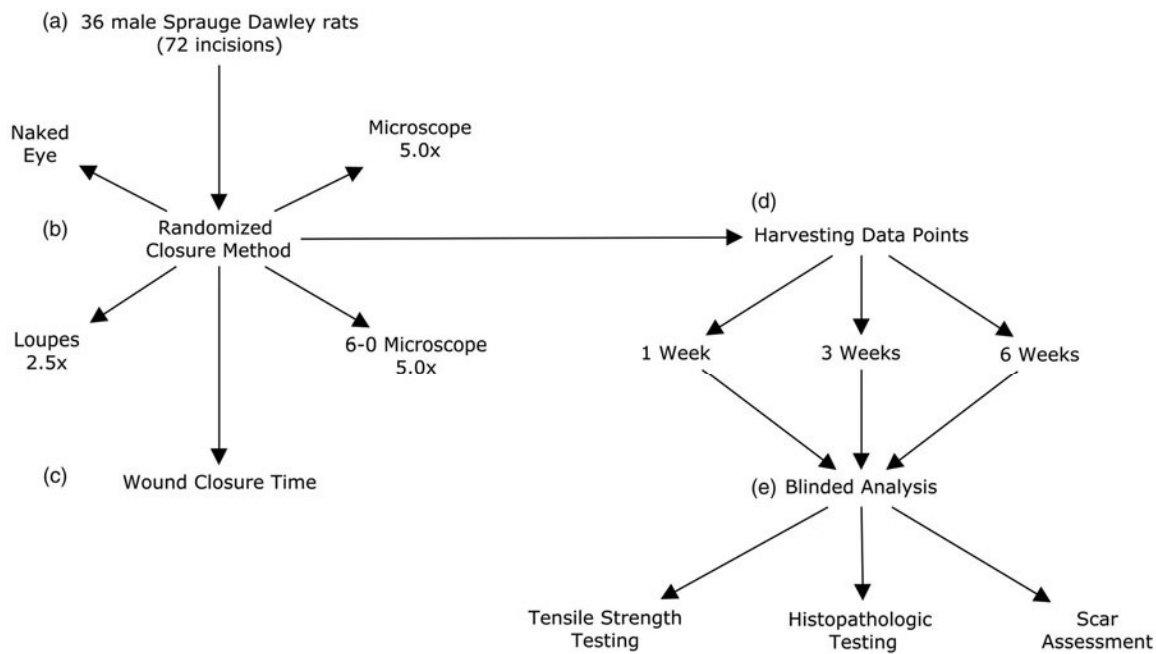


Figure 1. Experimental Design. (a) Two parallel incisions were made on either side of the spinal column of male Sprague–Dawley rats. (b) For each incision, closure type was randomized until there were six closures of each type at every time point. (c) Wound closure time for each pattern was recorded. (d) Rats were euthanized and incisions were harvested at 1, 3, and 6 weeks. (e) Sections of the harvested scars subsequently underwent blinded tensile strength testing, histopathologic, and scar assessment.

an impact on wound healing. The amount of time to close each incision was recorded. Post-operatively, animals were monitored in a post-surgical unit until completely recovered from anesthesia. Body weights were taken daily from post-operative day 1 through 5 and then weekly, as part of a pain and distress assessment. No animals met criteria for early termination from the study.

In groups of 12, the rats were then euthanized with carbon dioxide at 1 week, 3 weeks, and 6 weeks after the repair, thereby totaling six incisions per closure group in each time point. Three incisions were used for histological analysis and the other three were used for tensile strength measurements.

Gross/photographic analysis

Wounds were inspected daily for any evidence of complications. High resolution digital photographs of each incision were taken at the respective endpoints in each group. Using the photographs, the scars were rated according to Vancouver Scar Scale (VSS) by two independent, blinded plastic surgeons [19,20].

Tensile strength test

Tensile strength was measured using a method previously published by Lee et al. [21]. Briefly, harvested skin containing a healed incision was suspended with Kocher forceps. At one end, these forceps were connected to a freely suspended container. Water was slowly added to the container until any incision tear occurred (Figure 2). The weight of water necessary to achieve dehiscence was recorded.

Histological analysis

A 10 × 10 mm specimen from the center of each wound was harvested and saved in 10% neutral buffered formalin. Standard hematoxylin and eosin (H&E), and Masson's trichrome stains were



Figure 2. Harvested skin measuring one centimeter squared on either side of the incision was suspended by Kocher clamps. At one end (left of figure, not shown), a pulley system connected to the Kocher clamps to a hanging bucket. Water was slowly added to the container until incision dehiscence, and the weight of the water measured.

used for histological examination (Figure 3). A blinded pathologist performed quantitative analyses of specimen vascularity, fibroblast density, and epithelialization using Image J software [22]. For these analyses, five histological slides were selected randomly at each time point from each group and the measurements were performed on five microscopic fields, also selected at random.

Statistical analysis

The results from all the groups were compared using one-way analysis of variance. Significance was defined as $p \leq 0.05$. Analysis was done using STATA version 14.0 (Stata Corp, College Station, TX).

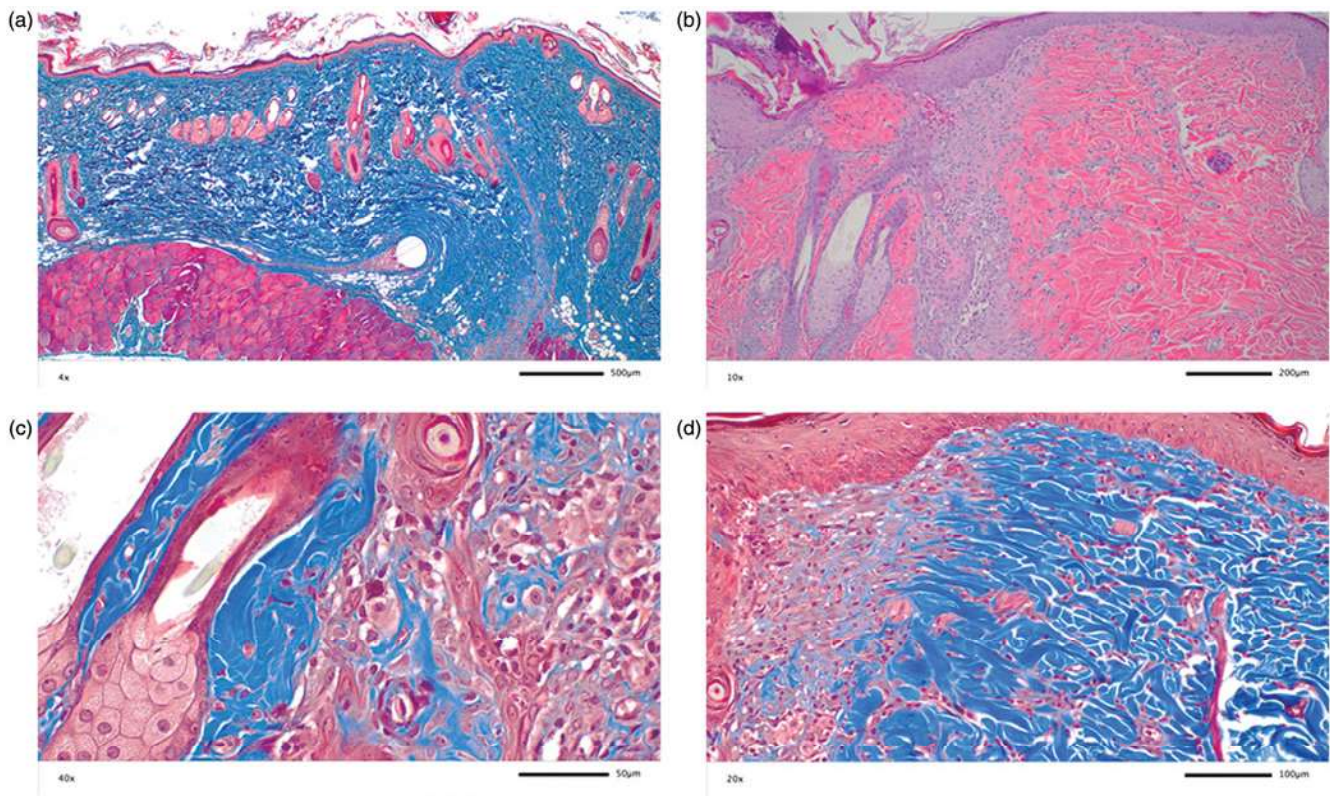


Figure 3. (a) Masson's trichrome stain at 4× magnification. (b) H&E stain at 10× magnification. (c) Masson's trichrome stain at 40× magnification. (d) Masson's trichrome stain at 20× magnification. Images demonstrate normal anatomy for healing.

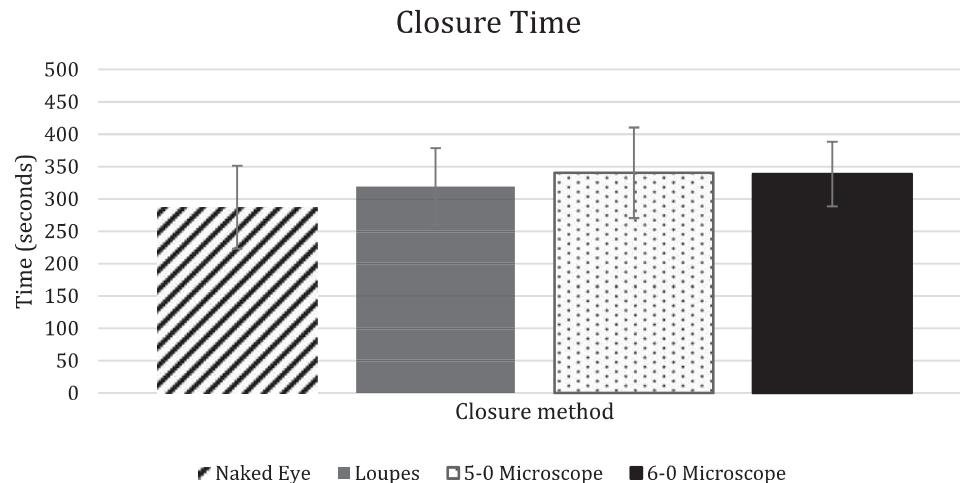


Figure 4. Average wound closure times for each closure method.

Results

Clinical evaluation

All of the rats survived until the end of the study. There were no instances of dehiscence or wound infection.

Wound closure time

The average wound closure time for naked eye closure, loupe closure, microscope closure and 6-0 with microscope closure was 287.3 ± 64 , 319.3 ± 59 , 340.4 ± 70 , and 338.4 ± 50 s, respectively. When groups III and IV were combined (groups closed using the microscope), the difference in time between naked eye closure

and microscope closure were statistically significant ($p < 0.05$) (Figure 4).

Observer evaluation

We found no significant difference in VSS ratings between the closure methods at any of the time points (Figure 5). When comparing between weeks, we found significant improvement in scars from week 1 to week 3 and to week 6, as might be expected by routine healing ($p = 0.01$ when comparing week 1 or 3 to week 6, $p = 0.05$ when comparing week 1 versus week 3). Figure 6 contains representative images from animals at each of the time points. Of note, a complete VSS analysis does include a tactile

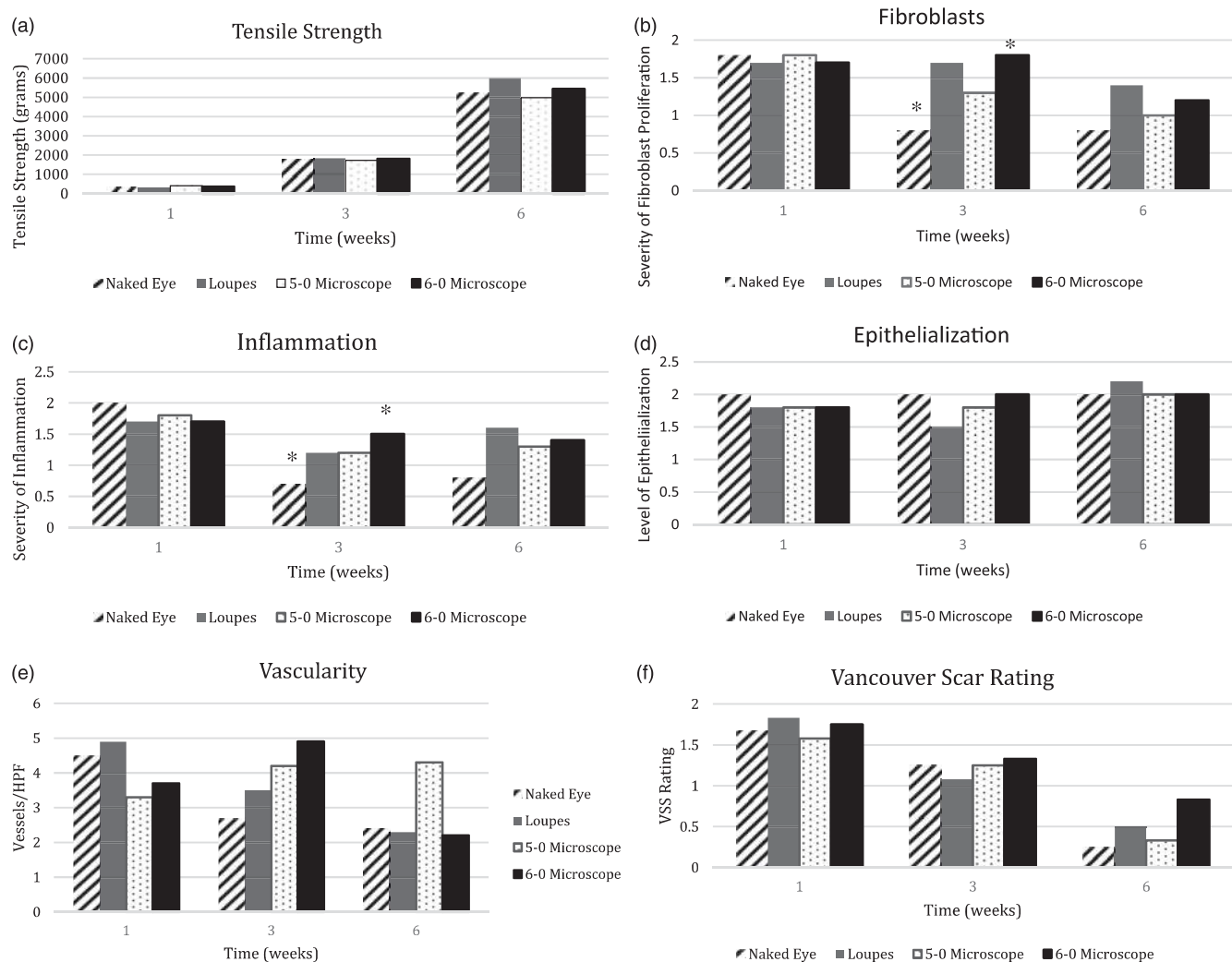


Figure 5. (a) Average tensile strength at each time point. (b) Average fibroblast proliferation at each time point. Fibroblasts were scored using the following scale: 0 = no fibroblast proliferation, normal collagen; 1 = mild fibroblast proliferation, mild collagen bundle irregularity; 2 = moderate to severe fibroblast proliferation. (c) Average severity of inflammation at each time point. Inflammation was estimated and scored using the following scale: 0 = no inflammation in specimen, 1 = less than 25 inflammatory cells, 2 = at least 25 inflammatory cells. (d) Average level of epithelialization at each time point. Epithelialization was estimated and scored using the following scale: 0 = no epithelialization, 1 = partially epithelialized, 2 = completely epithelialized. (e) Average vascularity at each time point. Vascularity was determined by the mean number of microvessels in five different high power fields on each slide. (f) Average Vancouver Scar Scale (VSS) assessment at each time point. Each scar was assessed by two blinded observers.

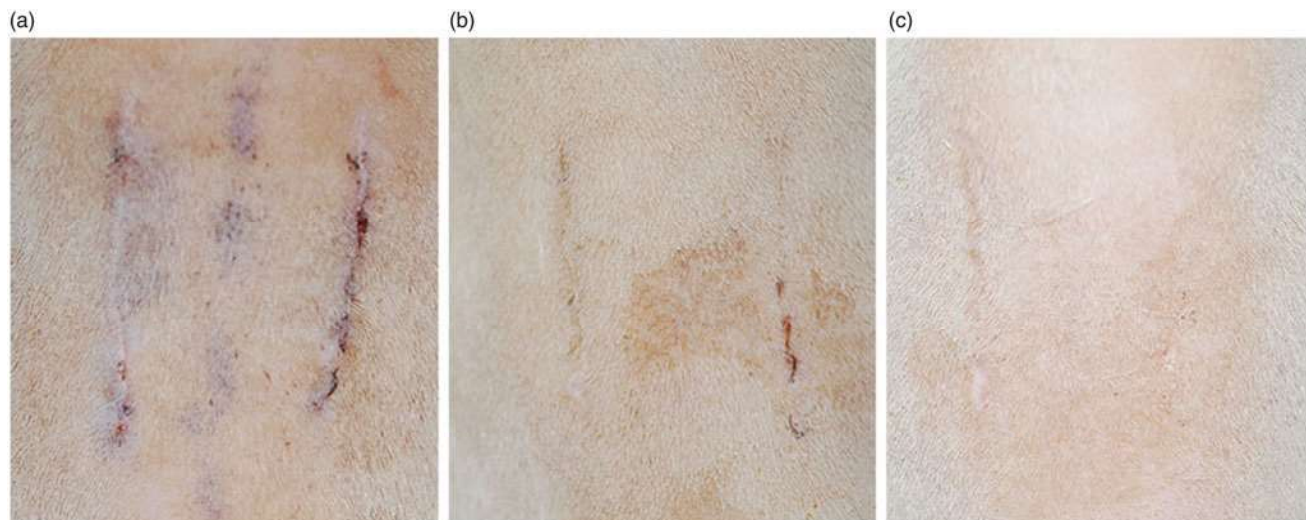


Figure 6. Representative images of scars at (a) 1 week, (b) 3 weeks, and (c) 6 weeks.

parameter, pliability. For the purposes of this study, we excluded this metric from analysis so that we could have two blinded plastic surgeons grade the scars based on high-resolution photographic images.

Tensile strength testing

The average tensile strength of each closure method increased progressively from week 1 to week 6, again as might be expected from routine healing (Figure 5). Tensile strength did not differ between closure methods at any time point ($p=0.9$ for week 1, $p=0.97$ week 3, $p=0.5$ week 6).

Histopathological evaluation

On histopathologic analysis, we saw a general trend of increased inflammatory markers with the microscopic closure methods in weeks 1 and 3. By week 6, however, there were no significant differences in any of the parameters (Figure 5). The only statistically significant comparisons were a greater number of inflammatory cells and fibroblasts in the 6-0 microscope closure group versus the naked eye closure group at week 3 ($p=0.05$ for inflammatory cells and 0.03 for fibroblasts, respectively). There was no significant trend or difference between groups in the rate of epithelialization at any time point.

Discussion

Our results show that all scars had an acceptable cosmetic outcome, with an average VSS rating of close to zero at 6 weeks for all closure methods. We saw no difference in tensile strength between closure methods. We did find more inflammatory markers with microscopic closure methods, likely related to the higher suture burden in these closures. However, these differences disappeared by 6 weeks. As one might expect, closures using the microscope took significantly longer to complete than loupe or naked eye closures.

Our study is the first to assess the impact of magnification on wound closure in a randomized, controlled fashion. Previous literature has been focused mainly on method of closure, including absorbable versus non-absorbable sutures [5], and closures accomplished with skin glue [14] or staples [11–13]. While smaller studies have often failed to show a difference between these closure methods, findings from large meta-analyses have suggested an increased rate of wound dehiscence with tissue adhesives versus sutures [14], and decreased rates of dehiscence when a sub-cuticular suture is utilized, versus when it is not [9]. A recent meta-analysis by Iavazzo et al. that included closures across different surgical disciplines demonstrated a decreased rate of infection with staple closures [12], although this finding was not borne out in the orthopedic [13] or cardiac surgery literature [11].

Yang et al. [7] evaluated the impact of suture size; this study found that smaller sutures were associated with increased inflammation. They attributed this finding to increased suture burden, similar to our study. Interestingly, this study found that increased inflammation correlated with a significant decrease in wound tensile strength. We suspect that the reason we did not see a similar difference in tensile strength was due to the fact that Yang et al. used 4-0 and 6-0 nylon sutures, which were removed ahead of strength testing, as opposed to absorbable sutures that remained in place.

Our results do not support the use of a microscope for routine skin closures. These closures took longer, and were not superior

with respect to wound strength or cosmesis. The costs of the extra time in the operating theater (and risk of longer general anesthetic), in addition to the cost of an operating microscope are clear deterrents to this method. We found equivalent results between naked eye and loupe closures, indicating that the choice between the two can be based on surgeon preference.

There are several limitations to this study. First, rats differ from humans in several aspects of wound healing. Unlike humans, rats have a panniculus carnosus muscle, which plays an important role in wound contraction and collagen synthesis. This causes wounds in rats to heal primarily by contracture as opposed to re-epithelialization, as seen in human wound healing. Re-epithelialization also occurs at very different rates in humans and rats due to the differences in the hair follicle (and, therefore, stem cell) density. The bearing of these facts on primarily closed wounds is unclear.

The main limitation of our study lies in the application of controlled study findings to the vast variety of wounds seen in clinical practice. Our wounds were short; small differences may be additive such that a significant difference would be seen with a longer surgical incision. Our wounds were all in one location; this study does not capture whether closure in certain anatomically complex areas (such as the ear or eyelid) would be better served with magnification. Our wounds were straight, clean surgical wounds. These wounds do not bear much resemblance to dirty, complex traumatic lacerations, where better tissue visualization may actually save time. In a multivariate analysis, Singer et al. demonstrated that sub-optimal cosmetic appearance was impacted by tissue trauma, incomplete wound opposition, electrocautery use, extremity location and wound width [1]. Because our incisions did not have any of these factors, the interplay of increased magnification and a cosmetically “high risk” wound is still unclear.

Conclusion

In conclusion, wound repair under magnification does not yield a significant difference in cosmesis or wound tensile strength. Moreover, there was a trend toward increasing inflammation with increased suture burden associated with microscopic wound repair. Closures using the microscope took significantly longer than those that did not. Based on our results, we believe that for the average, surgically created linear wound, magnification beyond that of 2.5× loupes is not supported.

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Disclosure statement

The views expressed in this material are those of the authors and do not reflect the official policy or position of the U.S. Government, the Department of Defense, or the Department of the Air Force. The animals involved in this study were procured, maintained, and used in accordance with the Laboratory Animal Welfare Act of 1966, as amended, and the Guide for the Care and Use of Laboratory Animals, National Research Council. The work reported herein was performed and funded under the United States Air Force Surgeon General-approved Clinical Investigation

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